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Effect of experimental diabetes on cholinergic, purinergic and peptidergic motor responses of the isolated rat bladder to electrical field stimulation or capsaicin

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Abstract

An attempt has been made to pharmacologically isolate cholinergic, P_2 purinoceptor-mediated and peptidergic (capsaicin-sensitive, tachykinin-mediated) contraction of the guanethidine-treated rat bladder detrusor preparation, in vitro. The effect of experimental diabetes was assessed on these types of contraction. Responses were evoked by electrical field stimulation (single shocks or 1 Hz for 30 s or 10 Hz for 40 s). Single shocks and 1-Hz stimulation were applied in the presence of (a) atropine (1 μM) or (b) P_2 purinoceptor antagonists (50 μM pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid) [PPADS] plus 100 μM suramin. Long-term electrical field stimulation (10 Hz for 40 s) (c) was applied with both atropine and the P_2 purinoceptor antagonists present in the organ bath. The effects of capsaicin (d) and ATP (e) were also studied. Three groups of experimental animals were used: streptozotocin-treated (50 $mg \cdot kg^{-1}$ i.p., 8 weeks before the experiment), parallel solvent-treated and untreated rats. (a) Responses to electrical field stimulation in the presence of atropine were reduced by half by PPADS plus suramin, but were resistant to capsaicin tachyphylaxis. They were enhanced in preparations taken from diabetic rats. (b) Contractions to electrical field stimulation in the presence of PPADS plus suramin were reduced by 2/3 by atropine, but were left unchanged by capsaicin or diabetes. (c) Contractions to long-term stimulation had a quick and a sustained phase. Especially the latter was inhibited by capsaicin tachypyhlaxis; it was also strongly reduced in preparations taken from diabetic rats. (d) Contractions to capsaicin (30 nM and 1 μM) were resistant to tetrodotoxin, strongly reduced by a combination of tachykinin NK_1 and NK_2 receptor antagonists, and slightly reduced in preparations from diabetic animals. Capsaicin (1 μM) had no acute inhibitory action on cholinergic or purinergic responses, nor did it cause relaxation in precontracted preparations treated with tachykinin receptor antagonists. (e) ATP-induced contractions were strongly reduced by PPADS plus suramin (50 plus 100 μM) and to a similar degree by 100 plus 200 μM, respectively. It is concluded that experimental diabetes selectively impairs peptidergic, capsaicin-sensitive responses (especially those that involve impulse conduction) in the rat detrusor preparation. The contractile response to electrical field stimulation that remains after atropine plus the P_2 purinoceptor antagonists has a yet unknown transmitter background.

Keywords: Urinary bladder, rat; Capsaicin; Cholinergic contraction; Purinergic contraction; Peptidergic response; Electrical field stimulation; Streptozotocin diabetes

1. Introduction

It has long been established that purinergic neurotransmission plays a role in the urinary bladder of various mammals (see Hoyle, 1994; Burnstock, 1997). It also became evident that motor responses that are mediated by other non-adrenergic, non-cholinergic (NANC) neurotransmitters also take place in this organ (Lundberg et al., 1984; Maggi et al., 1985). The occurrence of the tachykinin substance P has been demonstrated in capsaicin-sensitive sensory nerves of the bladder (Holzer et al., 1982; Sharkey et al., 1983). In functional experiments, Meini and Maggi (1994) found that a tachykinin-mediated, capsaicin-sensitive

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component is present in the field stimulation-evoked NANC contraction of the rat bladder detrusor muscle in vitro, provided that a sufficiently long train of stimuli is applied. Using selective antagonists for tachykinin NK_1 and NK_2 receptors, the above authors showed that these receptors are involved in the capsaicin-sensitive late contractile response; the conclusion follows that both substance P and neurokinin A probably are involved (Meini and Maggi, 1994). Another way of activating a capsaicin-sensitive response in the rat bladder in vitro is the administration of capsaicin itself. Capsaicin evokes an atropine-resistant contractile response that is sensitive to previous capsaicin treatment or tachykinin receptor antagonists, but not tetrodotoxin, a selective blocker of nerve axonal conduction (Maggi et al., 1985, 1991). This probably indicates that capsaicin stimulates sensory nerve endings (in a tetrodotoxin-resistant fashion) and releases tachykinins that in turn contract the smooth muscle.

Diabetic neuropathy encounters autonomic, somatomotor and sensory nerves. One of the manifestations of neuropathy is bladder dysfunction (Ellenberg, 1980). Capsaicin-sensitive nerves have been implicated in the alteration of bladder function in diabetes. With the rat detrusor, conflicting results are available as to the effect of experimental diabetes on the contractile action of capsaicin. Some studies indicate a decrease in the effect of capsaicin in preparations taken from diabetic rats (Kamata et al., 1992; Pinna et al., 1994), whereas others found no change in the motor effect of capsaicin in diabetes (Santicioli et al., 1987; Dahlstrand et al., 1992). As to other types of responses of the bladder, Santicioli et al. (1987) found a diminishment of the contractile action of electical field stimulation in diabetes. Especially depressed were responses due to low-frequency stimulation (0.1–5 Hz; without pretreatment). In the study of Dahlstrand et al. (1992) contractile responses to exogenous substance P were found enhanced in the bladder of diabetic rats, which might have been a result of denervation supersensitivity.

The aim of the present study was to assess the effect of experimental diabetes on the motor function of capsaicin-sensitive, as well as cholinergic and purinergic nerves of the rat urinary bladder in vitro. These components were relatively separated from each other by pharmacological means. Capsaicin-sensitive responses were studied by using capsaicin or long-train electrical stimulation, with muscarinic receptors for acetylcholine and P_2 purinoceptors blocked. Responses enriched in cholinergic and purinergic components were evoked with electrical stimulation in the presence of purinoceptor antagonists and atropine, respectively. The extent of the P_2 purinoceptor inhibition was assessed with the natural ligand, ATP. A pharmacological characterization of the capsaicin-induced response was performed. Moreover, it was also studied whether capsaicin-sensitive nerves can elicit a relaxant effect on the rat bladder or inhibit cholinergic or purinergic neuromuscular transmission.

2. Materials and methods

2.1. Streptozotocin treatment

Male Wistar rats were treated with streptozotocin (50 $mg \cdot kg^{-1}$) or its solvent i.p., 8 weeks before the experiment. They were kept in standard cages, with food and water ad libitum. Blood glucose level was determined 1 day before the experiment. Only those animals having a blood glucose concentration of at least 13 $mmol \cdot l^{-1}$ were included in the study.

2.2. In vitro experiments

Streptozotocin-treated, solvent-treated or untreated male rats weighing 370–500 g were killed by a blow on the occiput and exsanguination. The urinary bladder was excised and placed into a Silgard-coated Petri dish containing Krebs' solution of the following composition (mM): NaCl 119; $NaHCO_3$ 25; KCl 2.5; $MgSO_4$ 1.5; $CaCl_2$ 2.5; KH_2PO_4 1.2; glucose 11.

The bladders were halved sagittaly and made up for two detrusor preparations of approximate resting dimensions of 20 mm length and 2 mm width. Preparations were placed into 5-ml organ baths containing oxygenated (95% O_2 + 5% CO_2) Krebs' solution of 37 °C. They were connected to isotonic lever transducers (under a constant tension of 5 mN), for recording movements on a chart recorder.

Electrical field stimulation of the preparations was applied through a pair of platinum wire electrodes, placed into the bathing fluid at the top and the bottom of the organ bath, with a distance of 4 cm between them. Square-wave pulses were delivered by a high-power stimulator (Experimetria, Budapest, Hungary). Parameters of stimulation were 120 V amplitude, 0.1 ms pulse width; single pulses at a frequency of 0.1 Hz or trains of 1 Hz for 30 s or 10 Hz for 40 s.

Motor responses of the bladder were rendered relatively specific for cholinergic, purinergic or capsaicin-sensitive components by using the following drug treatments, atropine (1 μM) to block muscarinic acetylcholine receptor-mediated contractions, while leaving purinergic responses uninfluenced; the purinoceptor antagonists PPADS (50 μM) plus suramin (100 μM) to block purinoceptor-mediated contractions but leaving cholinergic responses uninfluenced; atropine plus PPADS plus suramin (concentrations as above) for inhibiting both cholinergic and purinergic responses without inhibiting capsaicin-sensitive contractions to long-term stimulation (10 Hz, 40 s) or capsaicin (30 nM or 1 μM). Guanethidine (3 μM) was present in the bathing fluid in all experiments to suppress possible adrenergic responses.

Unless indicated otherwise, the experimental protocol was as follows. Experiments were started after an equilibration period of 40 min. The effect of electrical field stimulation (single pulses, 1 Hz) was tested in the presence

of either atropine or PPADS plus suramin. The purinoceptor antagonists or atropine, respectively, were then added (contact time, 40 min), whereafter electrical field stimulation was repeated. The effect of long-term electrical stimulation (10 Hz, 40 s) was then tested. Following a recovery time of 15 min, the contractile action of capsaicin was established. Capsaicin (30 nM) was added for 3 min (the response peaked in approximately 1.5 min), then the concentration of capsaicin was elevated to 1 μM, for testing its effect and eliciting tachyphylaxis. The contractile response declined within 20–25 min. After 40 min following capsaicin administration, the long-term stimulation (10 Hz for 40 s) was repeated. This protocol of capsaicin treatment without rinsing was chosen because preliminary experiments have shown that rinsing increases the variability of the electrical field stimulation-induced responses.

Drugs used were capsaicin, guanethidine sulphate, suramin-Na, tetrodotoxin (Sigma), atropine sulphate (Merck), pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS; RBI). ((*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidine-3-yl]ethyl}-4-phenyl-1-azoniabicyclo[2.2.2]-octane chloride (SR 140 333), (*S*)-*N*-methyl-N [4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl) butyl]benzamide (SR48 968) (Sanofi Recherche, Montpellier, France), ω-conotoxin GVIA (Bachem).

Stock solutions of atropine (1 mM), guanethidine (1 mM), PPADS (20 mM), and suramin (10 mM) were prepared with isotonic saline, tetrodotoxin (500 μM) and ω-conotoxin GVIA (500 μM) with bidistilled water. SR 140 333 (1 mM) and SR 48 968 (1 mM) were dissolved in dimethyl sulfoxide. Capsaicin was dissolved in 96% ethanol to give a stock solution of 20 mM, which was also diluted with 96% ethanol if necessary. The final bath concentration of ethanol did not exceed 0.05% bath fluid.

2.3. Data analysis

The amplitude of the contractile responses was expressed as % of the maximal spasm evoked by KCl (80 mM) at the end of the experiments. Data are expressed as mean ± S.E.M. If not stated otherwise, *n* refers to the number of animals used. Statistical comparisons were made with Wilcoxon's signed rank test (for two related samples) or Mann–Whitney test (two independent samples). A value of $P < 0.05$ was taken to indicate statistically significant difference.

3. Results

Maximal contractions evoked by KCl (80 mM) in preparations taken from streptozotocin-treated rats, their solvent-treated controls or control rats of approximately the same weight did not significantly differ from each other.

3.1. Responses to electrical stimulation in the presence of purinoceptor antagonists

In control preparations (solvent-treated animals) in the presence of PPADS (50 μM) plus suramin (100 μM) both single electrical pulses and 30-s trains of stimuli delivered at 1 Hz elicited twitch-like and tonic contractions, respectively. Responses to 1 Hz stimulation needed approximately 20 s to reach their peak. Atropine (1 μM) significantly reduced these responses from 17.2 ± 1.9% to 5.8 ± 2.6% (single pulses) and from 45.9 ± 5.7% to 10.3 ± 2.6% (1 Hz; $n = 6$) (Fig. 1).

Under the same circumstances, bladder preparations taken from diabetic rats exhibited responses of similar height as did those from solvent-treated animals (Fig. 1). Responses also had similar time courses in both groups. Contractions in response to electrical field stimulation were also significantly reduced by atropine (1 μM), from 20.7 ± 1.0% to 10.3 ± 1.9% (single pulses) and from 49.2 ± 3.0% to 22.0 ± 3.2% (1 Hz; $n = 8$) in diabetic rats (Fig. 1).

In a separate set of experiments, the effect of capsaicin tachyphylaxis (1 μM capsaicin for 40 min, without rinsing)

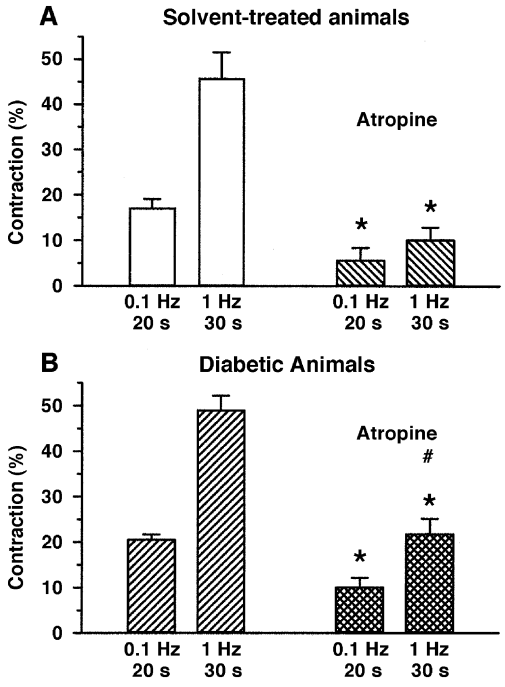

Fig. 1. Inhibition by atropine (1 μM) of the contractile effect of electrical field stimulation (single pulses delivered at 0.1 Hz and 30 s trains at 1 Hz) on bladder detrusor preparations taken from solvent-treated (A) and diabetic (B) rats. All preparations were pretreated with PPADS (50 μM) plus suramin (100 μM) and guanethidine (3 μM). The amplitude of the response is expressed as % of the maximal longitudinal spasm evoked by KCl (80 mM). Significant differences are shown above the columns: $^{*}P < 0.05$ (Wilcoxon's signed rank test) as compared to responses before atropine. $^{\#}P < 0.05$ (Mann–Whitney test) as compared with the solvent-treated group. Mean ± S.E.M. Contact time of atropine was 40 min. Number of animals: $n = 6$ (A), $n = 8$ (B).

or tetrodotoxin was tested on PPADS- plus suramin-treated preparations taken from untreated animals. Capsaicin tachyphylaxis caused no change in the amplitudes in contractile responses to single electrical pulses or trains of 1 Hz ($n=5$; data not shown). The effect of electrical field stimulation (single pulses or 1 Hz) was completely blocked by tetrodotoxin (1 μM; $n=4$, data not shown).

3.2. Responses to electrical stimulation in the presence of atropine

Contractile responses obtained in the presence of atropine (1 μM) were roughly similar in size to those received in the presence of the purinoceptor blockers (solvent-treated, $14.6 \pm 1.0\%$ with single pulses and $39.5 \pm 4.3\%$ at 1 Hz; $n=6$). At 1 Hz, these responses took approximately 10 s to reach their peak. Bladder strips prepared from diabetic rats exhibited significantly larger responses to field stimulation that did preparations taken from solvent-treated animals (Fig. 2). Apart from this, the shape of responses was similar in the two treatment groups. PPADS (50 μM) plus suramin (100 μM) caused an approximately 50% inhibition of these responses in both groups of preparations (Fig. 2), as well as in bladders taken from untreated animals ($n=5$). In the latter group, responses to field stimulation did not diminish if the concentrations of PPADS and suramin were enhanced from 50 to 100 μM and from 100 to 200 μM, respectively ($n=4$, data not shown).

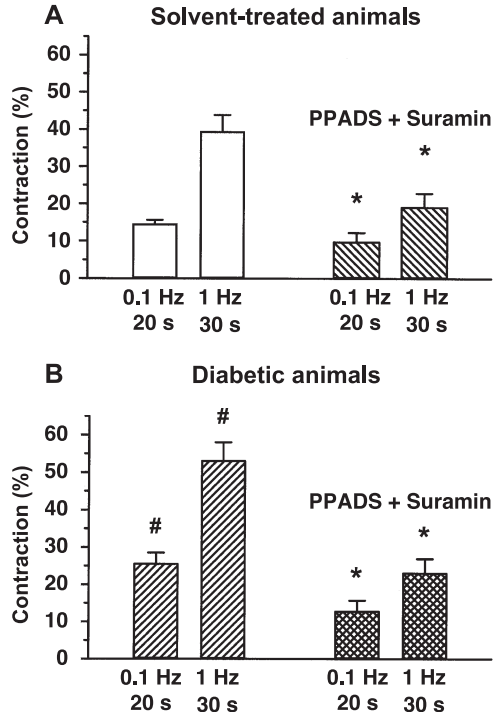

Fig. 2. Effect of PPADS (50 μM) plus suramin (100 μM) in rat detrusor preparations on the contractile effect of electrical field stimulation (single pulses delivered at 0.1 Hz and 30 s trains at 1 Hz), in the presence of atropine (1 μM) and guanethidine (3 μM). Preparations from solvent-treated (A) and diabetic (B) animals. *Significant difference ($P<0.05$) as compared to responses before PPADS plus suramin (Wilcoxon's test). #Significant difference ($P<0.05$) as compared with the solvent-treated group (Mann–Whitney test). Mean ± S.E.M. Number of animals: $n=6$ (A), $n=8$ (B).

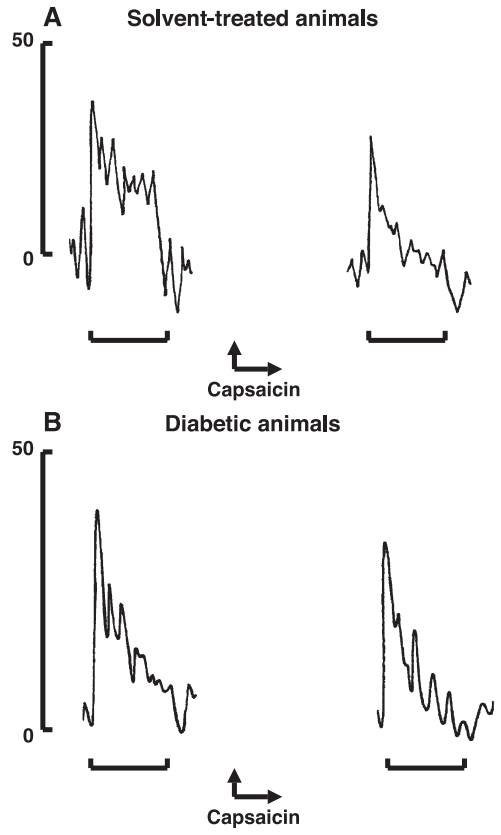

Fig. 3. Original tracings showing contractions of detrusor preparations taken from a solvent-treated (A) and a diabetic animal (B) in response to long-term electrical stimulation (10 Hz for 40 s; horizontal calibration). Effect of capsaicin tachyphylaxis (1 μM capsaicin, contact time: 40 min without rinsing). Vertical calibration, 50 % of maximal contraction with KCl (80 mM). Atropine (1 μM), guanethidine (3 μM), PPADS (50 μM) and suramin (100 μM) were present throughout.

In untreated animals, responses obtained with single pulses or 1 Hz were completely blocked by tetrodotoxin (1 μM; $n=6$, data not shown). Here again, capsaicin tachyphylaxis failed to influence responses to electrical stimulation (single pulses or 1 Hz; $n=6$, data not shown).

3.3. Effects of long-term electrical stimulation

In the presence of atropine (1 μM), PPADS (50 μM) and suramin (100 μM), a 40-s train of pulses delivered at 10 Hz typically caused a contractile response characterized by a quick and a sustained component (Fig. 3A). In bladders from untreated rats and without capsaicin pretreatment, the fast component of the response slightly decreased upon repeated stimulation (after 50 min, without rinsing) (from $34.6 \pm 6.4\%$ to $29.0 \pm 5.3\%$), while the sustained component (defined as tone at the end of the train of stimuli) proved reproducible ($29.1 \pm 5.0\%$ and $28.2 \pm 4.0\%$ upon

the first and second stimulation, $n=10$). Tetrodotoxin (1 μM) reduced the fast contraction by 91% and fully abolished the sustained one ($n=9$, data not shown). A pretreatment with capsaicin (1 μM for 40 min, without rinsing) slightly (by approximately 20%) decreased the quick component and strongly (by about 80%) reduced the sustained contraction in preparations from solvent-treated animals ($n=12$) (Fig. 3A).

In preparations taken from diabetic rats and before capsaicin, the sustained contraction to electrical stimulation proved approximately 60% smaller than in control preparations, whereas the initial contraction was moderately enhanced. In these preparations, capsaicin tachyphylaxis reduced the fast contraction to a similar extent as in solvent-treated preparations. The sustained response was practically abolished by capsaicin ($n=12$) (Fig. 3B; Table 1).

3.4. Contraction in response to capsaicin

In the presence of atropine, PPADS and suramin (concentrations as above), the contractile action of capsaicin (30 nM and 1 μM) was assessed with cumulative administration (3 min each) in 12 preparations taken from 12 solvent-treated animals and on 16 preparations taken from 12 diabetic rats. The effect of capsaicin was approximately 20% smaller in the latter than in the former group at both concentrations. The difference proved statistically significant at 1 μM of capsaicin (Fig. 4). Capsaicin (30 nM and 1 μM) completely lost its contractile action following an exposure to 1 μM capsaicin for 40 min without rinsing (tachyphylaxis, see above). This was true for untreated, solvent-treated or streptozotocin-treated animals ($n=4–6$, data not shown).

In a separate set of experiments on preparations taken from untreated animals, the pharmacology of the capsaicin-induced contraction was analysed. A sub-maximal concentration of 300 nM capsaicin was administered for 3 min. The effect of capsaicin was not influenced by tetrodotoxin (1 μM), or a combination of tetrodotoxin (1 μM) and ω-conotoxin GVIA (500 nM) (Table 2). Tachykinin receptor antagonists SR 140 333 (200 nM; for NK_1 receptors) or SR 48 968 (200 nM; for NK_2 receptors) failed to significantly affect the contractile response to capsaicin. A combination of SR 140 333 and SR 48 968 (200 nM both), however, caused a strong inhibition (approximately 80% reduction) (Table 2).

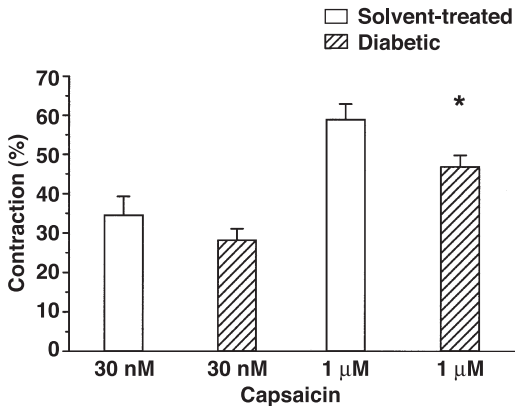

Fig. 4. Effect of experimental diabetes on the contractile action of capsaicin (30 nM and 1 μM, administered in a cumulative manner, contact time 3 min) on the detrusor muscle of the rat urinary bladder. Peak amplitudes of the longitudinal contractions are expressed as percentage of the maximal spasm due to KCl (80 mM). Data are presented as mean ± S.E.M. *Statistically different ($P<0.05$, Mann–Whitney test) from the solvent-treated group. Atropine (1 μM), guanethidine (3 μM), PPADS (50 μM) and suramin (100 μM) were present throughout. Number of experiments: $n=12$ (from 12 animals, solvent-treated), $n=16$ (from 12 animals, diabetic).

3.5. Lack of acute inhibitory effects of capsaicin

For detecting a possible hidden acute inhibitory effect of capsaicin on cholinergic or purinergic neurotransmission, 1 μM of capsaicin was administered 2 min before field stimulation (single electrical shocks and 1 Hz for 30 s, in

Table 1
Responses of the isolated bladder detrusor preparations to long-term electrical field stimulation (10 Hz, 40 s)

	Control, 10 Hz 40 s	Capsaicin (1 μM) tachyphylaxis	Number of animals
Solvent-treated animals			
Quick	26.1 ± 4.0	19.7 ± 2.7*	12
Sustained	25.2 ± 4.5	4.7 ± 1.6**	12
Diabetic animals			
Quick	40.3 ± 3.2◆	31.4 ± 4.0***,◆	12
Sustained	10.4 ± 2.3◆◆	1.3 ± 0.9**	12

Amplitudes of the quick and sustained phase of the responses were evaluated. Mean ± S.E.M. are given. Capsaicin tachyphylaxis was achieved by a 40-min administration of the drug, without rinsing. Responses are given as percentage of the maximal contraction provoked by KCl (80 mM). Statistically significant differences are shown by asterisks (responses before vs. in the presence of capsaicin; Wilcoxon's test) or diamonds (diabetic vs. solvent-treated animals; Mann–Whitney test). * or ◆—$P<0.05$, ** or ◆◆—$P<0.01$, ***—$P<0.001$.

Table 2
Effect of drugs on the capsaicin-evoked contraction of the rat urinary bladder detrusor muscle

Pretreatment	Contraction to capsaicin (300 nM)	*n*
–	41.6 ± 6.0	10
Tetrodotoxin (1 μM)	38.4 ± 5.9	6
Tetrodotoxin (1 μM) + ω-conotoxin GVIA (500 nM)	57.9 ± 6.6	6
SR 140 333 (200 nM)	34.9 ± 3.6	6
SR 48 968 (200 nM)	34.9 ± 3.6	6
SR 140 333 (200 nM) + SR 48 968 (200 nM)	8.6 ± 1.7*	6

Responses to capsaicin are given as percentage of the maximal contraction provoked by KCl (80 mM). Capsaicin was administered only once to each preparation. Mean ± S.E.M. are given.
Significantly different from the control group; *$P<0.01$; Mann–Whitney test.

the presence of PPADS plus suramin or atropine; $n = 4$ and 6, respectively). These experiments were carried out in preparations taken from untreated rats and a combination of SR 140 333 and SR 48 968 (200 nM each) was present throughout the experiments, for inhibiting the contractile action of capsaicin. Responses to field stimulation failed to show any change under the influence of capsaicin, as compared to a control cycle of stimulation, performed 30 min earlier. Likewise, we detected no relaxant effect of capsaicin (1 μM) in acetylcholine (1 μM)-precontracted preparations taken from untreated animals ($n = 5$). SR 140 333 and SR 48 968 (200 nM each) were also present in the bathing fluid in these experiments.

3.6. Effect of P_2 purinoceptor antagonists on the ATP-induced contraction

Different concentrations of ATP (one in each preparation) were administered before and 50 min after the application of PPADS (50 μM) plus suramin (100 μM). The contractile effect of ATP at any concentration was strongly suppressed by the antagonists (Fig. 5). The inhibition failed to increase if the concentration of each antagonist was doubled ($n = 4$ with 100 μM of ATP, data not shown).

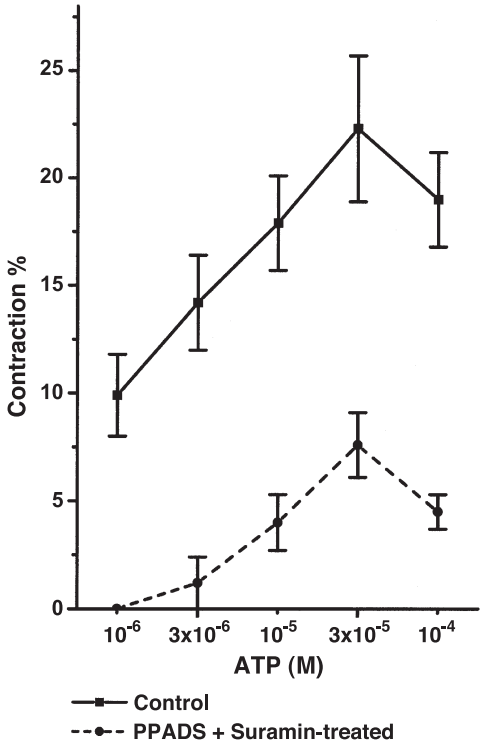

Fig. 5. Concentration–response curves for the contractile effect of exogenous ATP in the isolated rat urinary bladder detrusor preparation from solvent-treated animals, with or without (control) a mixture of PPADS (50 μM) plus suramin (100 μM). Contractions are expressed as percentage of the maximal spasm to KCl (80 mM). Mean ± S.E.M. are given. Atropine (1 μM) and guanethidine (3 μM) were present in the organ bath in all experiments.

4. Discussion

In this study, an attempt was made to pharmacologically separate the cholinergic, purinergic and peptidergic components of the contractile response of the rat bladder to electrical field stimulation, as well as to further characterize the contraction evoked by capsaicin in vitro and to assess the effects of streptozotocin-induced experimental diabetes on the electrical field stimulation and capsaicin-induced responses.

For inhibiting purinergic responses, we used a mixture of the P_2 purinoceptor antagonists PPADS (Lambrecht et al., 1992; Barthó et al., 1998) and suramin (Dunn and Blakeley, 1988). Similarly to α,β-methylene ATP tachyphylaxis (Brading and Williams, 1990; Luheshi and Zar, 1990), both suramin (Boselli et al., 1997; Tong et al., 1997) and PPADS (Tong et al., 1997) have been reported to inhibit contractions of the rat urinary bladder in response to electrical field stimulation. We used a combination of PPADS and suramin because our preliminary experiments suggested that the inhibition of electrical field stimulation-induced contractions by the mixture is slightly higher than that caused by either drug alone.

To avoid an interference with capsaicin-sensitive responses, we did not use stimulation frequencies higher than 1 Hz. In fact, capsaicin tachyphylaxis left these responses uninfluenced. Thus, it seems probable that the responses are mediated by autonomic, cholinergic and non-cholinergic efferents.

In the presence of PPADS and suramin, the response to electrical field stimulation to single electrical pulses or 1 Hz stimulation was to a large extent inhibited by atropine (a reduction by approximately 2/3), though a residual component of unknown origin was invariably observed. These neurons seem to be relatively resistant to damage due to experimental diabetes, since contractile responses of detrusor preparations taken from diabetic animals were not different from those of parallel controls receiving solvent treatment. A slightly smaller reduction by atropine of the responses to electrical field stimulation in the diabetic than in the solvent-treated group is in accordance with an enhancement of the atropine-resistant response in the diabetic group.

Responses in the presence of atropine are, to a considerable extent, purinergic in nature, as indicated by a significant reduction by PPADS plus suramin. Our previous experiments with PPADS indicated that this P_2 purinoceptor antagonist is apparently devoid of non-specific actions on the smooth muscle (Barthó et al., 1997, 1998, 2000). Approximately 50% of the response to electrical field stimulation was resistant to PPADS plus suramin. Comparable or somewhat stronger inhibitory effects of purinoceptor antagonists on the NANC contraction have been found by Tong et al. (1997) and Boselli et al. (1997). It is noteworthy that these authors detected a very strong inhibition of the contractile effects of P_2 purinoceptor agonists,

α,β-methylene-ATP and ATP by PPADS or suramin. Our experiments with exogenous ATP also indicate a strong (and insurmountable) inhibition by PPADS plus suramin, an effect that is not enhanced if the concentrations of the P_2 purinoceptor antagonists are doubled. The inhibition of the effect of exogenous ATP by the antagonists was stronger than their inhibitory effect on the electrical stimulation-evoked responses. Taken together, these data might indicate that the response we detect in the presence of atropine plus the P_2 purinoceptor antagonists is not purinergic in nature, although a participation of PPADS- and suramin-insensitive purinoceptors cannot be excluded.

There has been little doubt that the contractile response of the rat urinary bladder to capsaicin is mediated by tachykinins. Maggi et al. (1991) found that the tachykinin receptor antagonist spantide (a drug that acts mainly at NK_1 receptors) and the NK_2 receptor antagonists L-659 877 or MEN 10 376 selectively block the response to substance P and neurokinin A, respectively. Co-administration of these antagonists almost entirely inhibited the effect of capsaicin (1 μM), while either spantide or L-659 877 and MEN 10 376 alone was moderately effective. We confirmed this finding by using receptor subtype-specific antagonists SR 140 333 (200 nM) for NK_1 receptors and SR 48 968 (200 nM) for NK_2 receptors. These receptors apparently function in a supra-additive manner in the course of the capsaicin effect. Since the capsaicin-induced contraction is resistant to tetrodotoxin, it can hardly be assumed that tachykinins function as neuronal stimulants in this process, unless the terminal, tetrodotoxin-resistant part of neurons is activated. The finding that the response was also insensitive to ω-conotoxin GVIA (an inhibitor of the N-type Ca^{2+} channels) makes this latter possibility unlikely.

A pretreatment with 1 μM capsaicin abolished the contractile effect of capsaicin (30 nM–1 μM) indicating that this is a method applicable for the elimination of capsaicin-sensitive responses. The finding that capsaicin (1 μM) pretreatment also strongly suppressed the sustained contraction to long-term electrical field stimulation at 10 Hz, while leaving cholinergic or purinergic responses (to single pulses or 1 Hz stimulation) uninfluenced, indicates that this procedure has no appreciable non-specific smooth muscle depressant effect. We also found no evidence for a short-term relaxant effect of capsaicin in the rat bladder, even if the contraction induced was inhibited by tachykinin antagonists.

The capsaicin-sensitive sustained contraction to electrical field stimulation was markedly reduced in diabetic rats, while the effect of capsaicin was only moderately inhibited. Of what has been discussed above, it would follow that it is the capsaicin-sensitive neurons and not the smooth muscle that is damaged. Transmitter release from these neurons in response to electrical field stimulation is probably a more complex process than a release due to capsaicin, the former involving also fast Na^+ channels (as indicated by tetrodotoxin-sensitivity), then voltage-dependent Ca^{2+} channels and exocytosis. Impulse conduction in nerves is damaged by experimental diabetes (Patel and Tomlinson, 1999). On the other hand, the amount of sensory transmitters, at least of what is available for a release by capsaicin, is not probable to dramatically decrease, as indicated by the effectivity of capsaicin. The initial contraction to 10 Hz electrical field stimulation (in the presence of the P_2 purinoceptor antagonists and atropine) is, for the most part, of unknown origin; it is tentatively suggested that it has a similar transmitter background to the PPADS-, suramin- and atropine-resistant contractions seen at lower frequencies of stimulation.

In conclusion, a pharmacological separation of cholinergic, purinergic and capsaicin-sensitive peptidergic contractile responses of the rat bladder detrusor preparation has been performed. Contraction in response to electrical, but not to chemical activation of capsaicin-sensitive sensory nerves was strongly diminished in experimental diabetes.

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